Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 8129–8133, December 1985
Genetics

Tn*phoA*: A transposon probe for protein export signals

(gene fusions/alkaline phosphatase/Tn*5*)

COLIN MANOIL AND JON BECKWITH

Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115

Contributed by Jon Beckwith, July 31, 1985

ABSTRACT **We constructed a derivative of transposon Tn*5* that permits the generation of hybrid proteins composed of alkaline phosphatase (EC 3.1.3.1) lacking its signal peptide fused to amino-terminal sequences of other proteins. Such a hybrid gives alkaline phosphatase activity if the protein fused to alkaline phosphatase contributes sequences that promote export and thus compensate for the missing alkaline phosphatase signal peptide. Fusions to both a secreted periplasmic protein and a complex cytoplasmic membrane protein led to alkaline phosphatase activity. Tn*phoA* fusions should help localize export signals within the structure of a protein, such as a transmembrane protein, as well as identify new chromosomal genes for secreted and transmembrane proteins.**

Protein fusions have played a central role in molecular genetic studies of the mechanism of protein export in bacteria (1, 2). Most such studies have utilized hybrids containing amino-terminal sequences of an exported protein fused to the cytoplasmic protein β-galactosidase. One limitation in the use of such hybrid proteins is that the β-galactosidase moiety appears unable to pass through the bacterial cytoplasmic membrane (3).

To extend the utility of the gene fusion approach, Hoffman and Wright (4) constructed a set of plasmids that can be manipulated *in vitro* to fuse the gene for the *Escherichia coli* periplasmic protein alkaline phosphatase (EC 3.1.3.1) to different cloned genes. These plasmids encode an alkaline phosphatase missing its own signal sequence but retaining enough of the mature protein that highly active alkaline phosphatase can be generated in fusions. Unlike similarly constructed fusions with β-galactosidase, a variety of hybrids containing protein export signals attached to alkaline phosphatase were secreted and showed enzyme activity (4).

In this paper we present an extension of this approach that allows the ready isolation of fusions to alkaline phosphatase *in vivo*. We describe a transposon, Tn*phoA*, that can insert into a gene to generate fusions of alkaline phosphatase to amino-terminal sequences of the protein product of that gene. We present evidence that alkaline phosphatase export can be promoted not only by signal sequences of periplasmic proteins but also by sequences within complex cytoplasmic membrane proteins. This class of transmembrane proteins generally lacks cleaved signal sequences.

MATERIALS AND METHODS

Bacteria, Phage, Plasmids. Bacterial strains used were *E. coli* CC118 [*araD139* Δ(*ara, leu*)7697 Δ*lacX74 phoA*Δ*20 galE galK thi rpsE rpoB argE*$_{am}$ *recA1*], CC125 [CC118 *araC leu*$_{op}$ *sup-6 sup-9 lamB*], CC149 [MC1000 *phoR* (5)/F42 *lacI3*], and CC130 [MPh44 (5) *mutD5*]. The source of F42*ts*114 *lac* was ECO (6) and the source of F42 *lacI3* was JC5484 (7). Phage λ431 is *b*221 *c*I857 *rex*::Tn*5* (8). Plasmids pBR322 and pBR325 were described (9, 10). Plasmid pRI122 is a derivative of pBR322 carrying a modified IS*50*$_R$ (11). Plasmids pCH2, pCH39, and pCH40 are derivatives of pBR322 carrying *bla–phoA* fusions with *Pst* I linkers at the junctions between *bla* and *phoA* sequences (4). Plasmid pMLB1099 is a derivative of pBR322 carrying *lacZ* and was the gift of D. Jackson and M. Berman. Plasmid pB-4′ was made from pB-4 by eliminating the *Sma* I–*Hpa* I fragment of its Tn*phoA* insert. Plasmid pB-4′ΔSS was made by replacing the *Sca* I–*Bam*HI fragment of pB-4′ (carrying the amino-terminal coding region of the *bla* gene) with that from pTG2del1 (12).

Media and Cell Growth. Media were made according to Miller (13). Protein was radioactively labeled in cultures growing exponentially at 37°C in M63–glucose, supplemented with thiamin and each of the common amino acids except methionine, by exposing them to [^{35}S]methionine at 25–60 μCi/ml (1 Ci = 37 GBq) for 1–5 min.

Assay. Alkaline phosphatase activity of strains was assayed by measuring the rate of *p*-nitrophenyl phosphate hydrolysis by permeabilized cells (5). A background level of 2–3 units of activity (as defined in ref. 5) found in *phoA*$^-$ strains was subtracted from the values shown in Tables 1 and 2.

Antibody Precipitations. Protein extracts that had been boiled in the presence of $NaDodSO_4$ were immunoprecipitated with polyclonal antibody by using the protocol of Ito *et al.* (14).

$NaDodSO_4$/Polyacrylamide Gel Electrophoresis. Electrophoresis was carried out by using the protocol of Laemmli (15). Gels were fixed in 7.5% (vol/vol) acetic acid and treated with 1 M sodium salicylate. Dried gels were used to expose x-ray films. Protein bands were cut out of the gels, rehydrated with 100 μl of water, and shaken gently for 2 days at 42°C in a solution of 7.5% Protosol in Econofluor (DuPont) to elute radioactive material, which was quantitated by liquid scintillation analysis.

Isolation of Transposon Insertions into Small Multicopy Plasmids. To select transpositions of Tn*phoA*(op) (op indicating opal codon; see below) or Tn*phoA* into plasmids pBR322 or pBR325, an F′*lac* factor carrying the transposon to be inserted [F42*ts*114 *lac* zzf-1::Tn*phoA*(op) or F42 *lacI3* zzf-2::Tn*phoA*] was introduced into a *phoA*$^-$ strain (CC125 or CC118) carrying pBR322 or pBR325. Single colonies were resuspended in LB and dilutions were plated onto LB agar containing tetracycline (20 μg/ml) or ampicillin (200 μg/ml), kanamycin (300 μg/ml), and 5-bromo-4-chloro-3-indolyl phosphate (40 μg/ml). The high concentration of kanamycin in this medium enriches for cells carrying insertions of the transposon into the multicopy plasmid (16). After 2 days of incubation at 37°C, blue colonies (those with alkaline phosphatase activity) were streaked onto the same medium, and plasmid DNA was prepared from these cells after an additional day of growth at 37°C (17). Alternatively, plasmid DNA was made directly from the mixture of colonies that had grown on the agar with a high concentration of kanamycin. Plasmid DNA was used to transform *phoA*$^-$ recipient cells, with selection for blue transformant colonies growing on

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TYE agar containing kanamycin (30 μg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (40 μg/ml). Plasmids were analyzed for loss of gene function (e.g., ampicillin resistance) and by restriction mapping to determine sites of Tn*phoA* insertion.

Cell Fractionation. Cells were osmotically shocked to release periplasmic proteins, and then lysed (by a combination of lysozyme and freeze–thaw treatments) and sedimented to separate membrane and cytoplasmic fractions (refs. 18 and 19; S. Froshauer, personal communication). This fractionation protocol will be presented in detail elsewhere. In control fractionations using strain CC149, greater than 95% of alkaline phosphatase activity was recovered in the periplasmic fraction, 85–90% of the β-galactosidase activity was found in the cytoplasmic fraction, and 85–90% of NADH oxidase activity was recovered in the membrane fraction.

RESULTS

Construction of Tn*phoA*. We constructed a derivative of transposon Tn*5* able to fuse alkaline phosphatase (the *phoA* gene product) to amino-terminal sequences of proteins into whose genes it inserted (Fig. 1). This construction resulted in the placement of a DNA fragment encoding most of alkaline phosphatase (lacking only the coding region for the signal peptide and five additional amino acid residues) close to the left end of Tn*5*. When Tn*phoA* transposes into a gene with the *'phoA* orientation and translational reading frame the same as those of the target gene, a fusion protein should be produced.

Fusions of Alkaline Phosphatase to Proteins Encoded by Plasmids pBR322 and pBR325. To test the function of Tn*phoA*, we obtained derivatives of plasmids pBR322 and pBR325 into which the transposon had inserted. These plasmids were chosen because they encode β-lactamase (the *bla* gene product), a periplasmic protein that can substitute for an alkaline phosphatase signal sequence in export to give enzyme activity (4). We selected 26 insertions of Tn*phoA* into these plasmids that yielded active alkaline phosphatase in transformants (Table 1 and unpublished data). Eight of these insertions were indeed found to have generated *bla–phoA* fusions. Unexpectedly, however, 17 of the 26 active insertions fused alkaline phosphatase to the cytoplasmic membrane tetracycline resistance protein. A single fusion to the cytoplasmic protein chloramphenicol transacetylase and

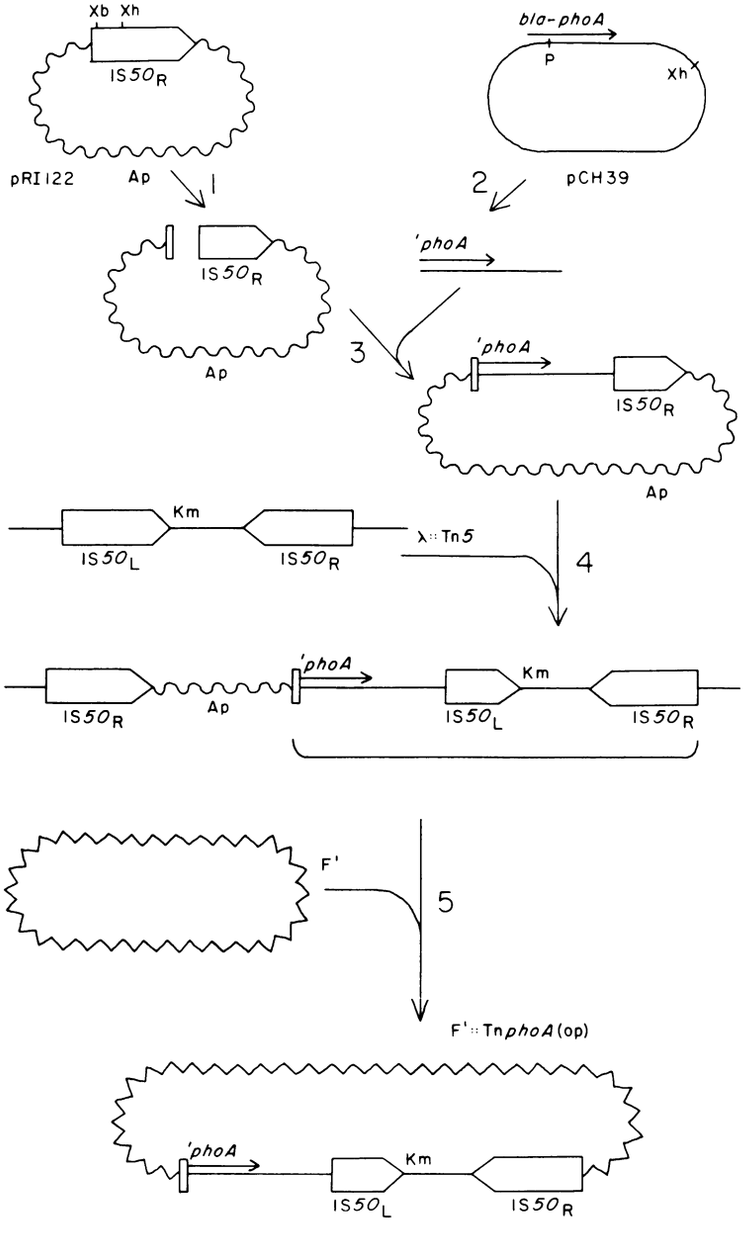

FIG. 1. Construction of Tn*phoA*. Tn*phoA* was made by inserting *phoA* sequences into IS*50* (steps 1–3), followed by recombination of this IS*50* derivative into the complete Tn*5* (steps 4 and 5). The construction steps were as follows: 1, sequential treatment of pRI122 with *Xba* I, *Bal*31 exonuclease, and *Xho* I; 2, sequential treatment of a pool of pCH2, pCH39, and pCH40 with *Pst* I, T4 DNA polymerase, and *Xho* I; 3, T4 DNA ligase treatment of a mixture of the purified fragments shown; 4, recombination of the plasmid resulting from step 3 at $IS50_L$ of λ::Tn*5* (λ431) to give a plasmid–phage cointegrate; and 5, transposition of Tn*phoA* (indicated by a bracket) from the cointegrate into an F′ factor (F42*ts*114 *lac*). Abbreviations: Xb, *Xba* I; Xh, *Xho* I; P, *Pst* I; Ap, ampicillin resistance; and Km, kanamycin resistance. Molecular cloning steps used standard protocols (17). Cointegrates resulting from the recombination diagrammed in step 4 were isolated from a phage lysate of λ431 grown on a strain carrying the plasmid resulting from step 3 by selection for cells resistant to ampicillin and kanamycin after infection and growth at 30°C. Insertions of Tn*phoA* into F42*ts*114 *lac* (step 5) were isolated after conjugation by selection for recipient cells becoming kanamycin resistant and capable of fermenting lactose but remaining ampicillin sensitive. One such derivative acted as a source of Tn*phoA* when insertions into plasmid pBR322 were selected and analyzed by restriction mapping. DNA sequence analysis (Fig. 3) revealed that pCH39 was the source of the *'phoA* fragment in Tn*phoA*. Plasmid and phage DNA are not drawn to scale.

Table 1. Protein fusions resulting from Tn*phoA*(op) and Tn*phoA* insertions

	Fusion plasmid*	Gene†	Parent plasmid	Size of hybrid protein, kDa		Alkaline phosphatase activity, units/OD_{600}
				Predicted‡	Observed§	
1.	b-101	*bla*	pBR322	74	75	28
2.	b-102	*bla*	pBR322	58	62	38
3.	b-103	*bla*	pBR325	49	48.5	3
4.	b-104	*bla*	pBR325	50	49.5	2.5
5.	t-101	*tet*	pBR322	52	50	38
6.	t-102	*tet*	pBR325	74	71	50
7.	t-103	*tet*	pBR325	86	82	27
8.	c-101	*cam*	pBR325	51	50	4
9.	B-1	*bla*	pBR322	56	55	328
10.	B-2	*bla*	pBR322	58	59	345
11.	B-3	*bla*	pBR322	66	70	356
12.	B-4	*bla*	pBR322	74	75	362
13.	T-1	*tet*	pBR322	50	48	82
14.	B-4′	*bla*	pBR322	—	75	300
15.	B-4′ ΔSS	*bla*	pTG2del1	—	75	<2
16.	G-1	*lacZ*	pMLB1099	85	96	<2
17.	G-2	*lacZ*	pMLB1099	89	101	<2
18.	G-3	*lacZ*	pMLB1099	104	120	<2
19.	G-4	*lacZ*	pMLB1099	148	157	<2

*Plasmid names beginning with lowercase letters (e.g., b-101 or t-102) derive from Tn*phoA*(op) insertion (lines 1–8), whereas those beginning with uppercase letters (e.g., T-1 or B-4′) derive from Tn*phoA* insertion (lines 9–19). Fusions were analyzed in strain CC125 (lines 1–8) or CC118 (lines 9–19).

†The *bla* gene encodes β-lactamase, the *tet* gene encodes the tetracycline-resistance protein, the *cam* gene encodes chloramphenicol transacetylase, and the *lacZ* gene encodes β-galactosidase.

‡Calculated from the position of the transposon insertion determined by restriction enzyme analysis, and assuming processing of the signal peptide from *bla–phoA* hybrid proteins.

§Determined for hybrid proteins precipitated by antibody to alkaline phosphatase and then subjected to electrophoresis.

showing very low enzymatic activity was also recovered. The electrophoretic migration of different hybrid proteins precipitated with antibody to alkaline phosphatase is shown in Fig. 2*A*. Each of the hybrid proteins (lanes 3–10) migrates as if its molecular weight is higher than that of wild-type mature alkaline phosphatase (lane 1). The low amount of hybrid proteins b-103 and b-104 relative to b-101 and b-102 probably results from the lower expression of β-lactamase from pBR325 than pBR322; pBR325 is missing one of the two *bla* promoters in pBR322 (20). The sizes of the different hybrid proteins agree well with those predicted from the positions of the Tn*phoA* insertions determined by restriction enzyme analysis (Table 1, lines 1–8).

Further evidence that *bla–phoA* hybrid proteins contain both β-lactamase and alkaline phosphatase sequences is provided by immunological studies. The proteins encoded by fusions B-2 and B-3 were precipitated by antibody to either alkaline phosphatase or β-lactamase (Fig. 2*B*).

Of 57 independently isolated insertions of Tn*phoA* into pBR325, at least 42 were in different positions as determined by restriction enzyme analysis (unpublished data). Tn*phoA* thus appears to retain the low DNA sequence specificity of insertion of Tn*5* (16).

Altering a Nonsense Codon of Tn*phoA*. Gene fusions generated by Tn*phoA* insertion were found to contain an opal (UGA) nonsense codon contributed by the $IS50_L$ sequence (21) at the junction between the target gene and *'phoA* (unpublished results). We had anticipated this possibility, and therefore the strain (CC125) we used to screen for hybrid protein activity (Fig. 2 and Table 1, lines 1–8) contained an opal suppressor mutation. We eliminated the opal codon from Tn*phoA*. This was done by growing a fusion plasmid (b-101) in a mutator strain (CC130), with selection for cells acquiring plasmid-linked, suppressor-independent expression of alka-

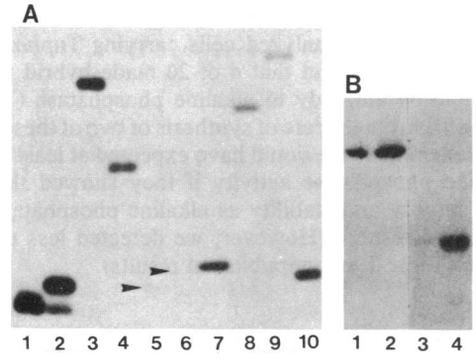

FIG. 2. Fusions of alkaline phosphatase to plasmid gene products. (*A*) Proteins precipitated by antibody to alkaline phosphatase after cultures were labeled 2 min with [^{35}S]methionine. Lanes: 1, mature alkaline phosphatase; 2, a mixture of precursor and mature alkaline phosphatase; 3, hybrid b-101; 4, hybrid b-102; 5, hybrid b-103; 6, hybrid b-104; 7, hybrid t-101; 8, hybrid t-102; 9, hybrid t-103; 10, hybrid c-101. The positions of weakly expressed hybrids b-103 and b-104 are indicated by arrows. (*B*) Proteins precipitated from cells producing hybrid B-3 by antibody to β-lactamase (lane 1) or alkaline phosphatase (lane 2), or from cells producing hybrid B-2 by antibody to β-lactamase (lane 3) or alkaline phosphatase (lane 4). The short hybrid encoded by fusion B-2 was less efficiently precipitated by antibody to β-lactamase than by antibody to alkaline phosphatase, presumably because it is missing antigenic sites recognized by the β-lactamase antibody.

line phosphatase activity. When the altered Tn*phoA* transposed to give new fusions, their activities also did not require suppressor function (unpublished results). The original transposon containing the opal codon is now referred to as Tn*phoA*(op), with the suppressor-independent derivative of it called Tn*phoA*.

DNA Sequence of the Tn*phoA* Left End. The DNA sequence of the left end of Tn*phoA* is shown in Fig. 3. There are 50 base pairs (bp) of DNA between the left end of Tn*phoA* and the beginning of the '*phoA* coding region. Of this sequence, 48 bp derive from $IS50_L$ (21) and 2 bp derive from the *Pst* I linker of pCH39 (4). The amino acid residues encoded by this 50-bp sequence are present at the fusion joint of every hybrid protein generated by Tn*phoA* insertion. The DNA sequence differs from that of $IS50_L$ by an A-to-G change at position 29 (21). This change eliminates the opal nonsense codon in frame with the alkaline phosphatase coding sequence. Tn*phoA* encodes all of alkaline phosphatase except the signal sequence and five amino acid residues of the mature protein (23).

Deletion of the Signal Sequence of a *bla–phoA* Fusion Protein. Kadonaga *et al.* (12) constructed a derivative of the *bla* gene of plasmid pBR322 in which the coding region for the 23 amino acid residue signal peptide is nearly precisely deleted, leaving only the region for the amino-terminal methionine and one other residue in its place. This protein is not secreted from the cytoplasm. When the normal *bla* amino-terminal coding region of fusion plasmid B-4' was replaced by that of the deletion mutant, cells carrying this plasmid (B-4'ΔSS) produced a hybrid protein the same size as those carrying the parental plasmid (unpublished results). However, cells carrying plasmid B-4'ΔSS were devoid of alkaline phosphatase activity (Table 1, line 15). The enzyme activity of a *bla–phoA* hybrid protein thus appears to depend on a functional β-lactamase signal sequence.

Isolation of *lacZ–phoA* Fusions. As an additional test of whether hybrids containing alkaline phosphatase require export to be enzymatically active, we sought to isolate fusions to the cytoplasmic protein β-galactosidase. However, with our usual method to select insertions of Tn*phoA* into a plasmid carrying *lacZ* (pMLB1099), we were unable to detect *lacZ–phoA* fusions giving alkaline phosphatase activity. Nevertheless, when we analyzed cells carrying Tn*phoA* insertions in *lacZ*, we found that 4 of 20 made hybrid proteins precipitable by antibody to alkaline phosphatase (Table 1, lines 16–19). From the rate of synthesis of two of these hybrid proteins examined, we would have expected at least 25 units of alkaline phosphatase activity if they showed the same specific activity and stability as alkaline phosphatase itself (unpublished results). However, we detected less than 2% this level (Table 1 and unpublished results).

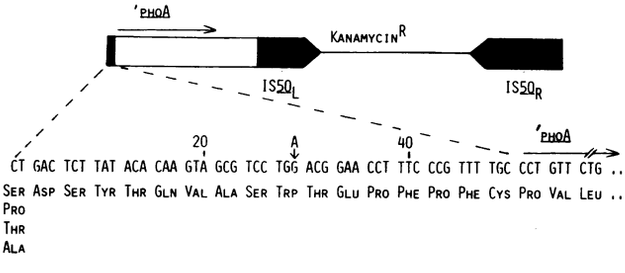

FIG. 3. Structure of Tn*phoA*, showing the nucleotide sequence of its left end and the deduced amino acid sequence. The nucleotide sequences of the junction fragments of two *malF–phoA* fusions generated by Tn*phoA* insertions were determined by the dideoxynucleotide chain-termination method after subcloning them in a phage M13 derivative (22).

Stability of Hybrid Proteins. The stabilities of different *bla–phoA* hybrid proteins were examined in pulse-chase experiments (unpublished results). The amount of the 75-kDa hybrid protein encoded by fusion B-4 decreased slowly as cells were incubated after labeling, showing a half-life of about 30 min. After 60 min, a second protein approximately the size of mature alkaline phosphatase (47 kDa) appeared. From the results of immunoprecipitation experiments using different antibodies, it appears that the smaller protein may be a degradation product of the full-length hybrid that has lost β-lactamase sequences (unpublished results). A second hybrid protein of 59 kDa encoded by fusion B-2 was more stable than that of fusion B-4, with a half-life of greater than 60 min.

The hybrid protein encoded by the derivative of fusion B-4 deleted of its signal sequence was extremely unstable, with a half-life of less than 6 min. In addition, unlike its parent, this hybrid protein showed no putative degradation product the size of mature alkaline phosphatase. Two *lacZ–phoA* fusion proteins were also relatively unstable. Talmadge and Gilbert (24) found that insulin antigen is also much less stable in the cytoplasm than in the periplasm.

Cellular Locations of Hybrid Proteins. The locations of different *bla–phoA* hybrid proteins were determined by cell fractionation (Table 2). The majority of the short hybrid protein encoded by fusion B-1 fractionated to the periplasm (line 1a). Although the full-length hybrid proteins encoded by three other *bla–phoA* fusions fractionated in part to the periplasm, they were also found in the membrane fraction (lines 2a, 3a, and 4a). The longer the hybrid protein, the greater the proportion of it found in the membrane fraction. The 47-kDa putative degradation products of these hybrid proteins fractionated mainly to the periplasm (lines 1b, 2b, 3b, and 4b). In contrast, the *bla–phoA* hybrid protein deleted for the *bla* signal sequence was not found in the periplasm and instead was found in the cytoplasm and membrane (line 5). Two *lacZ–phoA* fusion proteins were also not efficiently secreted, fractionating mainly to the cytoplasm (lines 6 and 7).

Long *bla-phoA* hybrid proteins may fractionate to the membrane because they lack carboxy-terminal β-lactamase sequences necessary for full solubility. In fact, long nonsense fragments of β-lactamase bind to the outside of the inner membrane (25).

Table 2. Fractionation of *bla–phoA* and *lacZ–phoA* hybrid proteins

	Fusion plasmid*	Protein size, kDa†	Protein distribution, %‡		
			Cytoplasm	Membrane	Periplasm
1a.	B-1	55	12	20	68
1b.	B-1	47	17	17	65
2a.	B-2	59	19	25	56
2b.	B-2	47	24	21	56
3a.	B-3	70	23	36	41
3b.	B-3	47	19	16	65
4a.	B-4'	75	12	55	33
4b.	B-4'	47	15	26	59
5.	B-4' ΔSS	75	50	46	4
6.	G-3	120	85	13	<2
7.	G-4	157	81	18	<2

*Plasmids B-1 to B-4' encode *bla–phoA* hybrids, while plasmid G-3 and G-4 encode *lacZ–phoA* hybrids. Proteins were fractionated from CC118 cells carrying F42*lacI3* and the plasmids listed (lines 1a–5) or CC118 alone and the plasmids listed (lines 6 and 7).

†Protein sizes were determined by electrophoresis. The proportions of molecules recovered in the 47-kDa bands were as follows: B-1, 27%; B-2, 12%; B-3, 25%; and B-4', 28%.

‡Radioactive protein was quantitated after precipitation by antibody to alkaline phosphatase and electrophoresis. Eighty-six percent of wild-type alkaline phosphatase radioactivity was recovered in the periplasm.

DISCUSSION

This report describes Tn*phoA*, a transposon that can fuse alkaline phosphatase lacking a signal peptide to amino-terminal sequences of proteins into whose genes it inserts. Results presented here support earlier work (4, 5) suggesting that alkaline phosphatase is not active unless it is exported from the cytoplasm. Therefore, for a fusion protein resulting from Tn*phoA* insertion into a gene to be enzymatically active, it appears likely that the product of that gene must contribute sequences that compensate for the missing signal peptide to promote export. Thus, Tn*phoA* acts as a probe for export signals in proteins into whose genes it inserts.

We found that insertions of Tn*phoA* in at least seven different positions in the gene for the periplasmic protein β-lactamase could give hybrid proteins with alkaline phosphatase enzymatic activity. The four of these *bla–phoA* hybrid proteins tested were found to be at least partially exported to the periplasm. The hybrid proteins were relatively stable, although most broke down slowly to give alkaline phosphatase-sized fragments.

Previous studies had indicated that alkaline phosphatase is inactive when sequestered in the cytoplasm (4, 5). Our results support this proposal. When the β-lactamase signal sequence was deleted from a *bla–phoA* hybrid protein, the hybrid was not exported to the periplasm and the cells no longer showed alkaline phosphatase activity. Furthermore, fusions to the cytoplasmic proteins chloramphenicol transacetylase and β-galactosidase gave very low or undetectable enzyme activity. Cytoplasmic alkaline phosphatase was found to be unstable, and our results do not distinguish whether its lack of activity is due solely to this instability or whether a stable cytoplasmic form would also be inactive. If inactivity is due to instability alone, it is possible that export of alkaline phosphatase is not the only mechanism that can stabilize it and thereby render it enzymatically active.

Hybrid proteins generated by Tn*phoA* insertion contain 17 amino acid residues at their fusion joints resulting from translation of Tn*5* and linker sequences (Fig. 2). We did not initially know how this junction sequence might affect the export behavior of hybrid proteins; however, our results indicate that it neither blocks export nor acts as an export signal.

Alkaline phosphatase fused to the tetracycline resistance protein can show enzymatic activity. This finding suggests that the alkaline phosphatase moiety of such hybrids is exported to the periplasm and that this cytoplasmic membrane protein contains sequences that can substitute for a signal sequence to promote export. It is not known whether the tetracycline resistance protein contains a cleaved signal sequence, although proteins with its presumed complex transmembrane structure generally do not (1, 26). Alkaline phosphatase fused to two other complex cytoplasmic membrane proteins (the MalF maltose transport protein and lactose permease) also gives enzyme activity (unpublished results).

The use of transposon Tn*phoA* combines the advantages of working with hybrid proteins able to be secreted (4) with the versatility of Tn*5* transposition in generating the hybrids (27–30). For example, fusing an exported protein to alkaline phosphatase provides a simple way to monitor the expression and localization of the protein, using the sensitive indicator media and enzyme assay available for alkaline phosphatase. Such a gene fusion can also be directly manipulated by using *phoA* genetic selections (31). In addition, by random insertion of Tn*phoA* into the chromosome, it should be possible to identify new genes encoding transmembrane and periplasmic proteins simply by their ability to give hybrid proteins with alkaline phosphatase activity. Our experiments show that fusions of alkaline phosphatase to complex transmembrane proteins can give active hybrids. Since alkaline phosphatase appears to be active only when it is in the periplasm, the positions of fusion joints of such active hybrids may help to identify regions of the membrane protein facing the periplasm. Thus, the use of Tn*phoA* may aid in the determination of the detailed transmembrane topology of such a protein. This analysis should also help to identify export signals in these complex transmembrane proteins. Finally, it is likely that Tn*phoA* can function in a number of bacteria other than *E. coli*, since its parent transposon Tn*5* shows a broad host range for transposition (27–30).

This work would not have been possible without the reliable advice of R. Isberg and C. Lee. We are also indebted to C. Hoffman and A. Wright for freely sharing results and plasmids; to D. Boyd, S. Froshauer, C. Gardel, H. Inouye, S. Michaelis, and R. Taylor for helpful discussions and demonstrations; to C. Lee for artwork; to T. Luna for media; and to A. McIntosh for help with the manuscript. C.M. is a Fellow of the Arthritis Foundation. This work was also supported by Grant MV-5M from the American Cancer Society.

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